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Case Report

# Prenatal diagnosis of holoprosencephaly associated with Smith–Lemli–Opitz syndrome (SLOS) in a 46,XX fetus

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ABSTRACT

*Objective:* To show the importance of measuring cholesterol precursor levels in amniotic fluid in all pregnancies with ultrasound features (such as holoprosencephaly) suggestive of Smith–Lemli–Opitz syndrome (SLOS), after exclusion of chromosomal anomalies.
*Case report:* A 28-year-old woman, gravida 1 para 0, performed chorionic villus sampling for fetal karyotyping at 13 weeks of gestation due to positive combined first trimester screening in a fetus with increased nuchal translucency and suspected holoprosencephaly. The result was normal – 46,XX. The diagnosis of alobar holoprosencephaly was confirmed at 15 weeks of gestation, and cardiac and limb defects were also identified. Thus, a syndromic cause was considered, specifically a chromosomal microdeletion syndrome or a monogenic entity such as SLOS. The latter was confirmed by measuring 7-dehydrocholesterol (7DHC) and 8-dehydrocholesterol (8DHC) in amniotic fluid. Molecular analysis of *DHCR7* gene identified a homozygous mutation in intron 8, c.964-1G>C, providing molecular confirmation for this diagnosis.
*Conclusion:* The differential diagnosis of holoprosencephaly is broad. Identification of the cause of holoprosencephaly aids in establishing the prognosis and is essential to ascertain the mode of inheritance for adequate genetic counseling.


## Introduction

Holoprosencephaly is a severe brain malformation that occurs between the 18th and 28th day of gestation and results from incomplete cleavage of the prosencephalon, affecting both the forebrain and the face [1]. The holoprosencephaly spectrum classically includes alobar, semilobar, and lobar forms, although there are no clear-cut defining features [2].

Genetic counseling about prognosis and recurrence risks for holoprosencephaly in the prenatal period is difficult due to extreme phenotypic variability and etiological heterogeneity [2]. In fact, although it is clear that neurodevelopmental outcome and mortality risk depend largely on the severity of holoprosencephaly, the prognosis also hinges on the etiology, which includes environmental factors and teratogens, chromosomal anomalies and monogenic syndromes (Table 1), and their associated features [1,3,4]. Furthermore, an etiological diagnosis is centermost to establishing the mode of inheritance and clarifying recurrence risks.

Here, we report a case of prenatal alobar holoprosencephaly due to Smith–Lemli–Opitz syndrome (SLOS), one of the rare causes of holoprosencephaly. The diagnosis was established based on high levels of 7-dehydrocholesterol (7DHC) and 8-dehydrocholesterol (8DHC) in amniotic fluid. Our aim is to show the importance of measuring cholesterol precursor levels in amniotic fluid in prenatal cases of holoprosencephaly after exclusion of chromosomal anomalies.

## Case presentation

We present the case of a 28-year-old primipara and her 39-year-old husband, both with no relevant past medical history. Routine fetal scanning at 13 weeks of gestation showed increased nuchal translucency of 4.23 mm (over the 99th centile, crown-rump length 62.3 mm), absent nasal bones, and intracranial abnormalities

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**Table 1**
Causes of holoprosencephaly.

| Category | Conditions |
|---|---|
| Environmental and metabolic | Maternal diabetes mellitus, alcohol,[a] retinoic acid,[a] cholesterol-lowering agents,[a] hypocholesterolemia[a] |
| Chromosomal | **Numerical**<br>Trisomy 13, trisomy 18, triploidy<br>**Structural**[b]<br>del(13q), dup(13q), del(18p), del(7)(q36), dup(3)(p24-pter), del(2)(p21), del(21)(q22.3) + several CNVs that include loci already known to be associated with holoprosencephaly |
| Syndromic monogenic[c] | Pallister–Hall syndrome, Rubinstein–Taybi syndrome, Kallmann syndrome, Martin syndrome, Hartsfield syndrome, "Pseudotrisomy 13 syndrome", **Smith–Lemli–Opitz syndrome**, Meckel syndrome, Genoa syndrome, Lambotte syndrome, hydrolethalus syndrome, facial clefts and brachial amelia, microtia-anotia and other anomalies, holoprosencephaly-agnathia spectrum disorders, caudal dysgenesis |
| Non-syndromic monogenic[c] | *SHH*, *ZIC2*, *SIX3*, *TGIF1*, *GLI2*, *PTCH1*, *DISP1*, *FGF8*, *FOXH1*, *NODAL*, *TDGF1*, *GAS1*, *DLL1*, *CDON* |

[a] Causality in humans not completely established.
[b] Large chromosomal deletions can be identified by conventional karyotyping, but molecular chromosomal investigation by chromosomal microarray analysis should always be considered as a first tier approach in malformed fetuses since it has the advantage of identifying submicroscopic abnormalities.
[c] With the exception of Smith–Lemli–Opitz that can be easily excluded prenatally by measuring 7DHC and 8DHC levels in amniotic fluid, most of these conditions require postnatal studies, including dysmorphological examination, fetal autopsy and, in some, molecular analyses. CNVs, copy number variations.

suggestive of holoprosencephaly. First trimester combined prenatal screening yielded a risk of 1/8 for trisomy 21 and 1/14 for trisomies 13 and 18. Fetal karyotyping in cells cultured from chorionic villus sampling was normal (46,XX). Fetal ultrasound was repeated at 15 weeks of gestation and documented alobar holoprosencephaly, subcutaneous edema, thickened nuchal fold, atrioventricular canal defect, and malformations of the hands and feet (Fig. 1). No renal, spinal or radial defects were observed.

The couple was then referred for genetic counseling for further etiological investigation. The review of ultrasonographic images did not suggest a specific diagnosis. However, most syndromic causes of holoprosencephaly are very difficult to diagnose on prenatal ultrasound alone and require a post-natal dysmorphological examination. In this case, the combination of holoprosencephaly, heart defect, hands and feet malformations and nuchal edema was in favor of a chromosomal microdeletion syndrome (to be investigated by chromosomal microarray analysis) or a monogenic entity. Among the latter, SLOS was the best fitting hypothesis based on the pattern of anomalies (Table 2). SLOS is one of the few monogenic syndromes that can be easily confirmed or excluded because the determination of 7DHC and 8DHC levels in amniotic fluid is a rapid and inexpensive analysis.

The pregnancy was terminated at 16 weeks of gestation but an amniocentesis was previously performed for 7DHC and 8DHC determination, and for cell culture in case further molecular or cytogenetic studies were needed.

The method for 7DHC and 8DHC quantification was previously described [5]. Briefly, an internal standard solution was added to 1 mL of amniotic fluid. Subsequently, saponification was achieved by adding potassium hydroxide in ethanol. The samples were then diluted with deionized water and the lipids were extracted with n-hexane. After centrifugation, the organic phase was transferred to a clean glass tube and dried using a gentle pressurized nitrogen stream. Finally, lipids were derivatized by addition of pyridine and BSTFA. Chromatographic analysis was performed using a QP 2010 Plus gas chromatograph–mass spectrometer (Shimadzu Co., Duisburg, Germany), equipped with an AOC-20i autosampler (Shimadzu Co.). A capillary column EquityTM-1 (15 m × 0.1 mm i.d., 0.1 μm film thickness) from Supelco (Bellefonte, USA) was used. The concentration of 7DHC and 8DHC in amniotic fluid was 30.4 μmol/L and 13.0 μmol/L, respectively (normal: undetectable).

Fetal autopsy showed a fetus small for gestational age (compatible with 13–14 weeks), with nuchal edema, holoprosencephalic face with a single-nostril, bilateral anophthalmia and low-set ears (Fig. 2), female genitalia with a prominent genital tubercle, imperforate anus, and bilateral postaxial polydactyly of hands and feet. The lungs were unilobar, there was only one ovary, and no thymus, kidneys, uterus and fallopian tubes were observed. These malformations are compatible with the diagnosis of SLOS.

Sequencing of *DHCR7* gene in fetal DNA revealed a homozygous pathogenic mutation in intron 8: c.964-1G>C (also described as IVS8-1G>C). This mutation, which is found in about 29% of SLOS patients, results in a null allele and confirms the diagnosis of SLOS [6]. Both parents were subsequently shown to be heterozygous for this mutation.

## Discussion

Holoprosencephaly is a heterogeneous malformation sequence of the midface and forebrain [6]. The clinical spectrum of severity

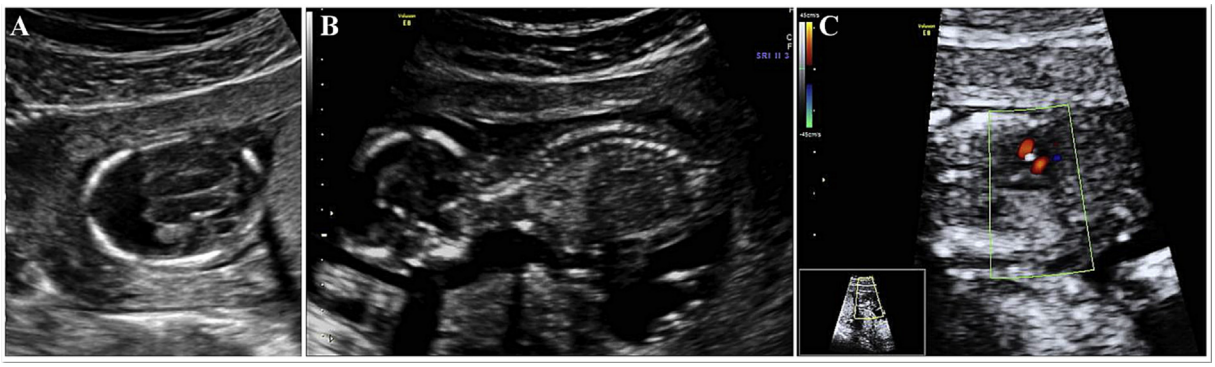


**Fig. 1.** Ultrasonography performed at 15 weeks of gestation showed (A and B) alobar holoprosencephaly, subcutaneous edema, thickening of nuchal fold, hands and feet malformations, and (C) atrioventricular canal defect.

**Table 2**
Genetic and clinical overview of non-chromosomal syndromic causes of holoprosencephaly.

| Etiology | Gene (inheritance) | Main clinical features |
|---|---|---|
| Pallister–Hall syndrome | *GLI3* (AD) | Hypothalamic hamartoma, bifid epiglottis, polydactyly. |
| Rubinstein–Taybi syndrome | *CREBBP*, *EP300* (AD) | Facial dysmorphism, broad and often angulated thumbs and great toes, microcephaly, short stature, intellectual disability. |
| Kallmann syndrome | Several genes (variable) | Congenital hypogonadotropic hypogonadism, anosmia or hyposmia (with hypoplasia or aplasia of the olfactory bulbs). |
| Martin syndrome | Unknown (AD) | Microcephaly, facial dysmorphism, cleft lip or palate, skeletal anomalies (namely of the feet and spine), chronic constipation, intellectual disability. |
| Steinfeld syndrome | Unknown (AD) | Holoprosencephaly, radial limb defects, heart defects, kidney malformations, absent gallbladder. |
| Hartsfield syndrome | *FGFR1* (AD or AR) | Holoprosencephaly, ectrodactyly. |
| Pseudotrisomy 13 syndrome | Unknown (AR) | Holoprosencephaly and polydactyly with a normal karyotype. |
| Smith–Lemli–Opitz syndrome | *DHCR7* (AR) | Facial dysmorphism, microcephaly, short stature, polydactyly and 2–3 toe syndactyly, hypogenitalism, cleft palate, cardiac defects, intellectual disability. |
| Meckel syndrome | Several genes (AR) | Encephalocele, large polycystic kidneys, polydactyly. |
| Genoa syndrome | Unknown (AR) | Holoprosencephaly, craniosynostosis. |
| Lambotte syndrome | Unknown (AR) | Brain malformations, microcephaly, intrauterine growth retardation, facial dysmorphism, early lethality. |
| Hydrolethalus syndrome | *HYLS1*, *KIF7* (AR) | Craniofacial dysmorphism, central nervous system, cardiac, respiratory tract and limb abnormalities. |
| Facial clefts and brachial amelia | Unknown (AR) | Brachial amelia, cleft lip, holoprosencephaly. |
| Microtia-anotia and other anomalies | Unknown (AD versus multifactorial) | External ear malformations, including microtia and anotia. |
| Complex agnatia holoprosencephaly | Unknown (AD versus multifactorial) | Absence or severe hypoplasia of the mandible, abnormal position of the ears, microstomia, holoprosencephaly. |
| Caudal dysgenesis | Unknown (unknown) | Variable anomalies of the caudal pole, including lower back and limbs, genitourinary and gastrointestinal anomalies. |

AD, autosomal dominant inheritance (confirmed or suspected); AR, autosomal dominant inheritance (confirmed or suspected).

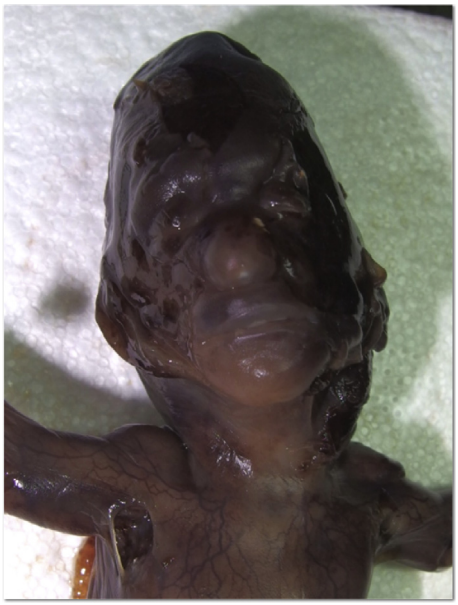

**Fig. 2.** Fetal autopsy showed holoprosencephalic face with a single-nostril, bilateral anophthalmia and low-set ears.

varies from the most severe alobar forms with cyclopia and absent septation of the cerebral hemispheres to microforms comprising microcephaly, hypotelorism, midfacial clefting, single central incisor, and other minor defects [1,3].

From an etiological standpoint, chromosomal anomalies are responsible for 24–45% of holoprosencephaly cases, most frequently numeric anomalies of chromosomes 13 and 18, and triploidy, but also structural anomalies, responsible for 10–20% and most commonly involving 13q, 18p, 7q36, 3p24-pter, 2p21, and 21q22.3 (in decreasing order of frequency) [4,7]. Between 18 and 25% of individuals with holoprosencephaly have a recognizable monogenic syndrome, such as SLOS [1,3].

SLOS is a multiple congenital anomaly syndrome caused by a defect in cholesterol metabolism resulting from 7-dehydrocholesterol reductase (DHCR7) deficiency [4,8]. The clinical spectrum is wide, ranging from minor birth defects and mild intellectual disability to prenatal or perinatal death due to severe organ malformations [9]. The typical pattern of abnormalities includes characteristic facial features, growth delay, microcephaly, polydactyly, syndactyly of the second and third toes, cleft palate, underdeveloped external genitalia in males and intellectual disability [10]. In the prenatal period, SLOS manifestations may include nuchal edema, intrauterine growth retardation, cleft palate, polydactyly, ambiguous genitalia and structural malformations of the kidneys, heart and brain. However, these findings are not specific for SLOS and may be observed in a variety of other congenital malformation syndromes [10]. Large series demonstrated that holoprosencephaly represents the most severe end of SLOS occurring in only about 5% [11,12]. *DHCR7* gene is the only gene in which mutations are known to cause SLOS [13].

Although several hypotheses have been proposed and the accumulation of sterol intermediates may have a role, the precise mechanism by which holoprosencephaly occurs in SLOS is not known [12,14]. Some theories suggest that low cholesterol and/or elevated 7DHC affect the function of one or several signaling proteins, including SHH, PTCH-1 and PTCH-2, or their receptors, such as smoothened [12]. Reduced cholesterol levels could impair *SHH* signaling through interference with the lamin B receptor, or damage transplacental cholesterol transport (directly or by interfering with megalin, the LDL receptor in embryonic neuroepithelium involved in maternal placental LDL transport), or alter embryonic plasma membrane functions and cell-to-cell interactions [13]. Concomitant mutations in *DHCR7* and one of the genes causing non-syndromic holoprosencephaly, including *SHH*, could be another mechanism [13]. Environmental and maternal factors are also expected to have a role [12,13].

It appears that DHCR7 activity and the severity of cholesterol deficiency alone cannot explain the development of

holoprosencephaly in SLOS. Nonetheless, prenatally, the clinical severity of SLOS correlates directly with 7DHC levels in amniotic fluid [14]. Although there is no specific genotype–phenotype correlation for the occurrence of holoprosencephaly in SLOS [15], most cases have carried two known or predicted null mutations, as is the case with this fetus.

SLOS follows an autosomal recessive pattern of inheritance, which entails a 25% recurrence risk for future pregnancies. The couple should be counseled accordingly and reproductive options, such as molecular prenatal diagnosis and preimplantation genetic diagnosis, should be offered.

The differential diagnosis of holoprosencephaly is broad (Table 1). Biochemical 7DHC and 8DHC measurements in amniotic fluid are cheap and easy analyses that can efficiently diagnose SLOS. We propose they should be performed in all pregnancies with suggestive ultrasound features (holoprosencephaly, acral and/or genital anomalies), after exclusion of chromosomal aberrations by karyotype or chromosomal microarray analysis.

## Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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